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16. ABSTRACT

Decamethrin is a synthetic pyrethroid insecticide that has been under investigation by the World Health Organization for use in some vector control programs. Decamethrin proved to be a highly toxic pyrethroid ester. The acute LD_{50} for adult female rats was 31 mg/kg by the oral route and 4 mg/kg by the intravenous route of administration. The LD_{50} was observed to be sex and age dependent, with higher values recorded for weanlings and males. Initial signs of decamethrin poisoning included profuse salivation and convulsive movements. Weakness, dyspnea, anorexia and staining of the fur were observed beyond the first day following compound administration. Absorption of decamethrin was rapid by the inhalation route and minimal by the dermal route of administration. No evidence of teratogenic activity was found in rats or mice at dose levels that produced marked maternal toxicity, and no persistent toxicity was observed in neonatal rats that received perinatal exposure to decamethrin. No mutagenic activity was detected in three different in vitro assays, with or without metabolic activation.

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TOXICITY STUDIES WITH DECAMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE

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Decamethrin is a synthetic pyrethroid insecticide that has been under investigation by the World Health Organization for use in some vector control programs. Decamethrin proved to be a highly toxic pyrethroid ester. The acute LD_{50} for adult female rats was 31 mg/kg by the oral route and 4 mg/kg by the intravenous route of administration. The LD_{50} was observed to be sex and age dependent, with higher values recorded for weanlings and males. Initial signs of decamethrin poisoning included profuse salivation and convulsive movements. Weakness, dyspnea, anorexia and staining of the fur were observed beyond the first day following compound administration. Absorption of decamethrin was rapid by the inhalation route and minimal by the dermal route of administration. No evidence of teratogenic activity was found in rats or mice at dose levels that produced marked maternal toxicity, and no persistent toxicity was observed in neonatal rats that received perinatal exposure to decamethrin. No mutagenic activity was detected in three different in vitro assays, with or without metabolic activation.

INTRODUCTION

Pyrethrins, natural products of the chrysanthemum flower, have been used extensively as insecticides for over fifty years (Griffen, 1973). Their relatively low mammalian toxicity and non-residual nature provide considerable environmental benefits. Williams (1973) reviewed the toxicology of natural pyrethrins and found no evidence for carcinogenicity, teratogenicity, or mutagenicity in test animals. In recent years synthetic pyrethroids have been extensively developed for insecticidal use. Miyamato (1976) reviewed the toxicology of synthetic pyrethroids and found

no evidence of carcinogenic, teratogenic, or mutagenic activity. However, he cautioned that possible adverse effects on humans have not yet been fully assessed. Decamethrin ([S]-α-cyano-*m*-phenoxybenzyl (1R,3R)-3-(2.2 dibromovinyl)- 2,2-dimethyl-cyclopropane carboxylate) was first described by Elliott *et al.* (1974) as part of a series of pyrethroids developed by the National Research and Development Corporation. Initial studies (Elliott *et al.*, 1974) indicated that decamethrin is a highly effective insecticide, although it possesses unusual mammalian effects (Barnes and Verschoyle, 1974). The acute intravenous lethal dose in young adult female rats was 2 to 2.5 mg/kg when administered as a solution in glycerol formal. Signs of poisoning included excessive salivation without lacrimation, rapidly followed by continuous jerking movements of the limbs, occasionally progressing to convulsions. The toxic effects were rapid in onset and brief in duration. Death occurred in some animals within 12 minutes of dosing; survivors were generally symptom-free within 6 hours. Although an acute oral LD_{50} could not be estimated, some rats died in the dose range of 25 to 63 mg/kg.

Decamethrin has been recommended for use in some vector control programs and is currently under investigation by the World Health Organization for such programs. The current studies were undertaken at the request of the WHO to further assess the acute, genetic, and perinatal toxicity of decamethrin.

MATERIALS AND METHODS

Technical decamethrin used in these studies was supplied by Roussel Uclaf, Romanville, France. Adult (3-4 months of age) male and female and weanling female (4-6 weeks of age) Sherman rats[1] were used in the acute oral, intravenous and dermal exposures. Male and female Sprague-Dawley rats[2] weighing 175 to 200 g were used in the inhalation experiments. Random-bred (60 days of age) CD-1 mice[3] and Sprague-Dawley rats[4] were used in the teratology experiments.

Acute Toxicity

In general, the procedure described by Gaines (1960) was followed in the acute oral toxicity studies. Decamethrin was dissolved in peanut oil and administered via gavage at a rate of 5 ml/kg body weight for the oral studies; in acetone and administered via a single injection into the tail vein at a rate of 0.313 ml/kg body weight for the intravenous studies, or, for the dermal studies, in xylene and administered at a rate of 3.2 ml/kg body weight to the back of animals after removal of fur with electric clippers. In the dermal studies, the animals were restrained in a Bollman-type cage (Bollman, 1948) to prevent their turning and licking the treated

[1]Simonsen Laboratories, Gilroy, CA.
[2]Center for Disease Control, Atlanta, GA.
[3]Charles River Breeding Laboratory, Inc., Wilmington, MA.
[4]Blue Spruce Farms, Altamont, NY.

area. After 5 days of restraint the rats were removed, washed with detergent and warm water, and returned to conventional cages. Control animals in the intravenous study received the vehicle alone at the same rate as test animals. Seven to 20 animals per dosage level were used. Several additional groups of animals were given lower dosages to determine the minimum toxic dose. Animals were observed over a 2-week period following dosing. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949).

Inhalation Toxicity

Aerosols were generated from 10 percent DMSO solutions using four DeVilbiss nebulizers. During the exposures, chamber samples were collected on nucleopore filters for analysis of chamber concentrations. The filters were weighed and gas chromatographic analysis of selected samples was performed using a Varian Model 3740 Gas Chromatograph equipped with a 50 x 0.2 cm stainless-steel column packed with 5 percent OV-101 on Chromosorb G. The column and injector temperature was 250°C, the electron capture detector temperature was 300°C, and nitrogen carrier gas flow was 31 ml/min. The gas chromatographic analysis indicated that approximately 60 percent of the gravimetric sample was decamethrin; the other 40 percent presumably, was solvent. Ten animals of each sex per dosage level were used.

DEVELOPMENTAL Toxicity

Prenatal Studies. Day 1 of pregnancy was recorded upon demonstration of sperm in the vaginal smear.

Decamethrin was dissolved in corn oil and administered by gastric intubation at doses of 12.0, 6.0, 3.0, or 0 mg/kg during days 7-16 of gestation for the mice and 5.0, 2.5, 1.25, or 0 mg/kg during days 7-20 of gestation for the rat. Maternal weight on day 6 was used for the calculation of doses. Intubation volume was 0.2 ml for both species. All animals were treated between 8 and 10 a.m. Control animals received vehicle alone.

Mice and rats were sacrificed on day 18 and 21 of gestation, respectively. The uterus was examined *in situ* for the number of live fetuses as well as for signs of dead or resorbed fetuses. The uterus was then removed and weighed. The maternal weight gain was calculated as the difference between the overall weight gain during pregnancy and the gravid uterus weight. The fetuses were removed from the uterus, blotted, weighed as a group, examined for gross abnormalities, and divided equally for fixation in either Bouin's solution or 65 percent alcohol. The former were necropsied while the latter were stained with alizarin red S and examined for skeletal abnormalities and maturity.

Postnatal Studies. An additional group of pregnant rats was housed individually and intubated with dosages of either 5.0, 2.5, or 0 mg/kg

from day 7 of gestation to day 15 of lactation in the manner described above. These dams were allowed to deliver and rear their young. Litters were reduced at birth to four individuals of each sex, weighed weekly, and examined for the development of eye opening, startle reflex (a jerking motion of the entire body in response to a mechanically produced click) and air righting (ability to land on all four feet when dropped upside down from a height of 25 cm). The litters were weaned on day 22 post-partum and the males were discarded. The females continued to be weighed weekly, and in the sixth post-natal week they were tested in a circular open field[5] similar to that described by Broadhurst (1957). All test procedures in these postnatal studies were conducted on an experimenter-blind basis.

Statistics. Data in the tables are presented in terms of the mean plus or minus the standard error. Statistical tests utilized in this study included the Mann-Whitney *U*-test for comparisons among the treatment groups and Jonckheere's test (Jonckheere, 1954) for dose-response analysis. For all comparisons, except those for open-field behavior (where the individual was used as the unit of comparison,) the litter was regarded as the fundamental unit of comparison.

Mutagenicity

Salmonella typhimurium. The *S. typhimurium* stains were kept at 4°C on minimum medium plates, supplemented with a trace of biotin and an excess of histidine. The plates with the plasmid-carrying strains contained ampicillin (25 μg/ml) to insure stable maintenance of plasmid pKM101. New stock culture plates were made every two months from single colony reisolates that were checked for their genotypic characteristics and for the presence of the plasmin. For each experiment, an inoculum from the stock culture plates was grown overnight at 37°C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures were shaken for 3 to 4 hours to ensure optimal growth. Some carcinogenic chemicals are inactive unless matabolized to active forms; therefore the S-9 activation system described by Ames (1975) was used.

To a sterile test tube placed in a 43°C heating block, the following were added in order: 1) 2.00 ml of 0.6 percent agar (containing 0.05 M histidine and 0.05 M biotin), 2) 0.05 ml of indicator organism, 3) 0.50 ml of S-9 activation mixture (optional), and 4) 0.05 ml of a DMSO solution of the test chemical. For negative controls, steps 1), 2), and 3) and 0.05

[5]The open field was constructed of Plexiglas and was 72 cm in diameter with walls 30 cm high. A 25 watt light blub was positioned 80 cm above the center of the field. A cheesecloth curtain which circumscribed the field from this height provided a uniform environment to the animals. The floor was marked off into three concentric circles: a central area, a middle ring marked into 6 "squares," and external ring marked into 12 "squares." All "squares" were of the same approximate area. Testing was conducted for a period of 4 minutes on two consecutive days. To initiate a test, the subject was placed in the center circle and the following information was collected: latency to leave the circle, defecations, urinations, rearings, and activity (one unit of activity was defined as having a minimum of three legs in a square at the same time). Activity occurring in the external ring was distinguished from that occurring in the two center rings. The floor was wiped clean following each test.

ml of DMSO were used. For positive controls, 2-anthramine and 9-aminoacridine were used.

The mixture was stirred gently and then poured onto minimum agar plates. After the top agar set, the plates were incubated at 37°C for 2 days. The number of *his*$^+$ revertant colonies was recorded.

Saccharomyces cervisiae D3. The *S. cerevisiae* tester strain was stored at -80°C. For each experiment, the tester strain was inoculated in 1 percent tryptone and 0.5 percent yeast extract and grown overnight at 37°C with aeration. The suspension was then centrifuged and the cells resuspended at a concentration of 10^8 cells/ml in 67 mM phosphate buffer (pH 7.4). To a sterile test tube the following were added: 1) 1.30 ml of the organisms, 2) 0.50 ml of either the S-9 activation mixture or buffer, and 3) 0.20 ml of the test chemical in DMSO, or DMSO alone in the negative control. A positive control of 1,2,3,4-diepoxybutane was included.

The suspension was incubated at 30°C for 4 hours on a roller drum. The sample was diluted serially in sterile physiological saline and 0.2 ml was spread on tryptone-yeast agar plates. The 10^{-5} dilution was spread on 3 plates, and the 10^{-3} dilution was spread on 5 plates. The plates were incubated for 2 days at 30°C, followed by 2 days at 4°C to enhance development of the red pigment of adenine-deficient homozygosity. Plates were scanned for red sectors (mitotic recombinants) and surviving colonies. The number of recombinants was calculated per 10^5 survivors.

Escherichia coli WP2. A procedure similar to the *Salmonella* assay was used to measure the reversion of WP2 to tryptophan independence. However, the minimal agar was supplemented with 1.25 g of Oxoid nutrient broth per liter to provide each plate with the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical. No additional tryptophan was added to the top agar.

RESULTS

Acute Toxicity

Acute toxicity data, expressed as LD_{50} values, are summarized in Table 1. In general, females were more sensitive than males, and weanlings were more sensitive than adults via the intravenous route but less sensitive via the oral route. With cutaneous administration, absorption of the decamethrin appeared negligible, since 800 mg/kg applied to the skin failed to produce any signs of toxicity. This dosage was 80 times the minimal dosage required to elicit effects with the oral route.

Based upon the assumptions that a 200 g rat has a minute volume of 80 ml/min, that 100 percent of the material is absorbed in the lungs, and that 60 percent of the inspired material is decamethrin, the LD_{50} via the inhalation route is 36 mg/kg for adult males and 28mg/kg for adult females.

An acute inhalation toxicity value for decamethrin was also calculated based on differing exposure durations. An LCT_{50} (a value derived

TABLE 1. Acute Toxicity of Decamethrin to Rats

Route	Strain	Sex	Age	LD_{50} (mg/kg)	95% Confidence Limit of LD_{50} (mg/kg)	Lowest Dose Tested (mg/kg)	Mimimum Toxic Dose (mg/kg)
Oral	Sherman	M	Adult	52	46-58	30.00	30[a]
Oral	Sherman	F	Adult	31	29-34	5.00	10[b]
Oral	Sherman	F	Weanling	50	42-60	7.50	15[b]
Intravenous	Sherman	F	Adult	4	2.9-5.3	1.57	1.57[a]
Intravenous	Sherman	F	Weanling	1.8	1.5-2.1	0.78	0.78[a]
Dermal	Sherman	F	Adult	> 800	—	800.00	—[c]
Inhalation[d]	Sprague-Dawley	M	Adult	940 mg/m^3	—	—	
Inhalation[d]	Sprague-Dawley	F	Adult	785 mg/m^3	—	—	

[a]Moderate to severe salivation and convulsions.

[b]Mild salivation.

[c]No signs of toxicity at 800 mg/kg.

[d]Based upon exposure time of 120 to 150 minutes.

by multiplying the atmospheric concentration necessary to kill 50% of the population by the exposure time) of 1.12×10^5 mg min/m^3 for male rats and 1.00×10^5 mg min/m^3 for female rats was obtained from atmospheric, mortality, and exposure duration data. The LCT_{50} value is useful in estimating and comparing acute toxicity data over a wide range of concentrations and duration of exposures, and is probably more significant than the LD_{50} or chamber concentration normally presented when acute inhalation data is generated.

Clinical signs of toxicity were similar for all routes of exposure. The rate of onset was most rapid with intravenous administration. The first observed effect following intravenous dosing was scratching of the neck region in the area of the salivary glands. Salivation, often accompanied by irregular breathing, was the first observed sign for the oral and inhalation routes. Ataxia, which rapidly progressed to convulsive movements, developed soon thereafter. Salivation was profuse at the higher dose levels, with the saliva being clear at first then becoming bloody and wetting the entire underside of the animal. Except in a few animals the primary signs (salivation and convulsions) did not persist into the second day. Secondary signs (weakness, dyspnea, anorexia, and staining of the fur) were observed beyond the first day. A recurrence of the primary signs appeared 24-28 hours after dosing in 6 of 57 animals surviving oral doses of 35-55 mg/kg, and in one rat the salivation and convulsions recurred about 44 hours after dosing. Recovery from the oral dosage was most rapid in weanling females (most were normal after 20 hours, and all within 44 hours) and slowest in adult males (some residual effects were evident up to 5 days after dosage). Recovery from the intravenous dosage was complete within about 6 hours.

Gross examination of the animals that died showed congestion of the lungs and adrenals. In some animals flatulence of the stomach and intestines was observed. No gross pathological changes were observed in animals necropsied 14 days after exposure.

Developmental Toxicity

Mouse. Administration of decamethrin to pregnant mice resulted in a dose-related ($p < 0.001$) reduction in maternal weight gain during pregnancy (Table 2). Pregnant dams in the high dosage group (12.0 mg/kg/day) gained 58 percent less weight than did those in the control group. No dose-related occurrences of maternal mortality were observed, but most animals in the high dosage group and some in the middle dosage group did become convulsive soon after dosing. These convulsions, although similar to those previously described for the rat, occurred at higher dosages than in that species. The overall reduction in fecundity observed in this experiment was due to effects seen in the first replicate assay. Eleven of 15 control females were pregnant in this assay versus 4/14, 4/15, and 2/14 in the low, middle and high dosage groups, respectively. No similar effects were noted in the second assay. In fact, the lowest fecundity (50 percent) was noted in the control population of

TABLE 2. Prenatal Effects of Decamethrin in the CD-1 Mouse

	Dose (mg/kg/day)			
	0	3.0	6.0	12.0
Maternal Observations:				
No. Inseminated	30	30	30	30
No. Died	3	2	4	5
No. Pregnant	17	15	10	9
Avg. Maternal Weight Gain (g)	4.5 ± 0.6[c]	3.7 ± 0.5	2.8 ± 0.7	1.9 ± 0.5
Avg. Maternal Liver/Body Wt. Ratio	6.5 ± 0.1	6.8 ± 0.1[d]	6.7 ± 0.1	6.6 ± 0.3
Fetal Observations:				
Avg. No. Implants	12.5 ± 0.6	13.1 ± 0.6	12.8 ± 0.7	12.7 ± 0.8
Avg. % Mortality	10.3 ± 2.6	10.7 ± 2.7	15.9 ± 4.3	10.9 ± 2.7
Avg. Fetal Weight (g)	0.94 ± 0.03	0.96 ± 0.05	0.87 ± 0.05	0.92 ± 0.04
Avg. No. Sternal Ossification Centers	5.5 ± 0.1	5.3 ± 0.2	4.7 ± 0.6	5.4 ± 0.3
Avg. No. Caudal Ossification Centers	3.3 ± 0.2	3.3 ± 0.2	2.7 ± 0.4	3.5 ± 0.6
Avg. % Supernumerary Ribs	13.3 ± 6.2[b]	23.4 ± 5.2[e]	47.1 ± 12.4[e]	28.2 ± 7.0[d]
Visceral Abnormalities[a] Enlarged Renal Pelvis	6.5/11.8	1.2/6.7	3.8/10.0	0/0

[a]Represented as % fetuses affected/% litters affected.
[b]Significant dose-related response, $p < 0.01$.
[c]Significant dose-related response, $p < 0.001$.
[d]Significantly different from control value, $p < 0.05$.
[e]Significantly different from control value, $p < 0.01$.

TABLE 3. Prenatal Effects of Decamethrin in the Sprague-Dawley Rat

	Dose (mg/kg/day)			
	0	1.25	2.5	5.0
Maternal Observations:				
No. Inseminated	32	29	32	37
No. Died	0	2	1	0
No. Pregnant	28	25	28	26
Avg. Maternal Weight Gain (g)	45.6 ± 2.6[a]	44.4 ± 2.1	43.9 ± 2.7	36.5 ± 2.0
Avg. Maternal Liver/Body Wt. Ratio	4.4 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.1
Fetal Observations:				
Avg. No. Implants	11.5 ± 0.5	10.9 ± 0.7	12.6 ± 0.4	12.1 ± 0.5
Avg. % Mortality	13.8 ± 5.2	5.4 ± 1.6	8.6 ± 4.4	11.8 ± 5.0
Avg. Fetal Weight (g)	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
Avg. No. Sternal Ossification Centers	5.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
Avg. No. Caudal Ossification Centers	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.8 ± 0.2
Avg. % Supernumerary Ribs	7.2 ± 2.4	22.6 ± 5.2	10.0 ± 2.8	18.0 ± 4.8
Visceral Abnormalities[b]				
Enlarged Renal Pelvis	16.5/46.2	28.0/64.0	24.2/63.0	17.8/52.0
Rhinencephaly	0.7/3.8	0/0	0/0	0/0

Significant dose-related response, $p < 0.01$.

[b]Represented as % fetuses affected/% litters affected.

TABLE 4. Postnatal Effects of Decamethrin in the Sprague-Dawley Rat

		Dose (mg/kg/day)		
	Sex	0	2.5	5.0
No. Females Delivered/ No. Inseminated	—	10/12	8/14	11/14
Avg. Litter Size	—	10.8 ± 0.6	12.0 ± 0.9	11.7 ± 0.4
Survivorship (%)				
d8	—	100	100	97.7
d22	—	100	98.4	94.1
Weight (g)				
d1	M+F	6.4 ± 0.1	6.2 ± 0.2	6.2 ± 0.2
d22	M	56.1 ± 1.7[a]	53.1 ± 1.2	52.8 ± 1.2
	F	52.8 ± 1.9[a]	51.2 ± 1.1	49.0 ± 1.3
d43	F	158.4 ± 4.2	154.5 ± 3.3	152.8 ± 4.3
Startle Reflex (age)	M	13.8 ± 0.4	13.8 ± 0.3	14.5 ± 0.8
	F	13.2 ± 0.3	14.0 ± 0.3	13.7 ± 0.3
Eye Opening (age)	M	15.9 ± 0.6	16.1 ± 0.3	16.1 ± 0.3
	F	15.9 ± 0.3	16.3 ± 0.3	15.8 ± 0.2
Righting Reflex (age)	M	17.6 ± 0.2	18.6 ± 0.4	18.2 ± 0.3
	F	18.1 ± 0.5	17.5 ± 0.3	18.8 ± 0.3
Open Field Behavior	F			
No. Tested		20	16	22
d1				
Rearings		25.8 ± 2.4	25.7 ± 3.6	26.4 ± 1.9
Defecations		2.9 ± 0.6[a]	2.7 ± 0.7	1.5 ± 0.3
Total Activity		112.9 ± 7.0	101.7 ± 7.9	109.3 ± 4.5
Internal Activity (%)		6.2 ± 1.0	9.3 ± 1.4	8.7 ± 1.1
d2				
Rearings		14.7 ± 1.9	16.1 ± 2.7	18.2 ± 1.4
Defecations		1.5 ± 0.5	1.7 ± 0.5	1.4 ± 0.3
Total Activity		86.3 ± 8.0	69.6 ± 8.8	87.7 ± 7.6
Internal Activity (%)		8.6 ± 1.5	8.5 ± 1.5	10.4 ± 1.5

[a]Significant dose-related response, $p < 0.05$.

this assay. No effects were observed on the number of implantation sites, fetal mortality, or fetal weights, or in the number of sternal and caudal ossification centers. A significant ($p < 0.01$) dose-related increase in the occurrence of supernumerary ribs was observed. No other dose-related skeletal or visceral anomalies were observed in decamethrin-treated mouse fetuses.

TABLE 5. *In Vitro* Assays of Decamethrin with *Salmonella typhimurium*—Experiment 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	–		16	4	17	24	149
	+		10	5	21	20	153
Positive controls							
9-Aminoacridine	–	100		498			
2-Anthramine	+	2.5	121		222	1030	339
Decamethrin	–	10	16	7	12	20	175
	–	50	18	7	17	29	175
	–	100	14	6	8	38	167
	–	500	23	4	12	31	154
	–	1000	13	7	12	21	150
	+	10	16	14	14	31	108
	+	50	11	6	14	23	127
	+	100	9	8	10	31	126
	+	500	8	7	15	30	123
	+	1000	10	6	14	23	175

Rat. Administration of decamethrin to pregnant rats also resulted in a dose-related reduction ($p < 0.01$) in maternal weight gain during pregnancy (Table 3). Animals in the high dosage group (5.0 mg/kg) gained only 80 percent of the control value. This dose was sufficient to produce a mild salivation evident for up to four hours after dosing in approximately 50 percent of the animals. No effects were observed on the number of implantation sites, fetal mortality, fetal weight or number of sternal and caudal ossification centers.

Administration of decamethrin to rat dams during pregnancy and lactation produced no effect on parturition, litter size, or pup viability (Table 4). Neonatal weights at birth were similar for all groups, but a

dose-related depression in growth was observed during the pre-weaning period. The differences, which were significant ($p < 0.01$) on day 15 post-partum, decreased ($p < 0.05$) at weaning (day 22) and were absent by day 36 post-partum. Thus, the maximum depression in weight was noted on the last day of compound administration (day 15 post-partum) and rapidly diminished upon cessation of dosage. The decrease in weight

TABLE 6. *In Vitro* Assays of Decamethrin with *Salmonella typhimurium*—Experiment 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	–		50	8	24	44	145
	+		20	22	28	53	126
Positive controls							
2-Anthramine	–	2.5				49	
	+	2.5	516		1107	1430	1434
9-Aminoacridine	–	100		218			
Decamethrin	–	50	53	11	25	25	146
	–	100	49	6	21	12	143
	–	500	46	12	7	24	117
	–	1000	43	10	14	29	145
	–	2500	47	6	18	31	140
	–	5000	48	13	20	30	134
	+	50	23	18	28	41	108
	+	100	21	14	42	36	128
	+	500	12	10	31	58	130
	+	1000	13	17	28	37	120
	+	2500	11	12	38	35	153
	+	5000	14	10	35	42	130

did not affect the age at which the startle or righting reflexes occurred, nor did it affect the age at eye opening. Female offspring, when tested in an open field at six weeks of age, showed no differences in activity or exploratory measurements. Thus, the early diminution in preweaning weight appears to have little effect on those morphological or behavioral parameters measured.

Mutagenesis. Decamethrin was not mutagenic in assays with *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, or TA100, either in the presence or absence of a rat-liver homogenate metabolic activation system (Tables 5 and 6). An apparent increase in revertants with strain TA100 plus metabolic activation at the highest dose level (Table 5) was not confirmed when additional doses were tested in a subsequent experiment (Table 6).

Decamethrin was not mutagenic in assays with *E. coli* WP2 (Table 7), nor did it increase mitotic recombination in *Saccharomyces cerevisiae* D3 (Table 8).

TABLE 7. *In Vitro* Assays of Decamethrin with *Escherichia coli* WP2

Compound	Micrograms/ Plate	Metabolic Activation	Tryptophan Revertants Per Plate	
			Expt. 1	Expt. 2
DMSO Control		–	37	37
		+	31	31
2-Anthramine	2.5	–	93	93
Decamethrin	10	–	25	28
	50	–	24	34
	100	–	27	32
	500	–	21	20
	1000	–	20	33
	10	+	24	37
	50	+	40	40
	100	+	38	37
	500	+	42	36
	1000	+	28	32

DISCUSSION AND CONCLUSIONS

The acute LD_{50} values obtained in this experiment are in agreement with those of Barnes and Verschoyle (1974). The toxicity as calculated for the inhalation route is of the same order of magnitude as the oral treatment, suggesting a rapid absorption and distribution. In contrast, the data from the dermal exposures indicate that decamethrin is poorly absorbed by this route, as is true for other synthetic pyrethrins (Miyamoto, 1976). Signs of toxicity following decamethrin administration differed from those previously described for synthetic pyrethrins (Miyamoto,

TABLE 8. *In Vitro* Assays of Decamethrin with *Saccharomyces cerevisiae*

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($\times 10^{-7}$)	Percent	Per ml ($\times 10^{-3}$)	Per 10^5 Survivors
EXPERIMENT 1						
Negative control	—		6.9	100	2	2.9
	+		5.5	100	1	1.8
Positive control 1,2,3,4-Diepoxybutane	–	0.04	6.4	93	570	890
Decamethrin	–	0.1	7.0	101	1	1.4
	–	0.5	4.9	71	4	8.2
	–	1.0	6.7	97	1	1.5
	–	5.0	3.5	51	1	2.9
	+	0.1	7.2	131	3	4.2
	+	0.5	6.6	120	2	3.0
	+	1.0	6.9	125	1	1.5
	+	5.0	6.3	115	6	9.5
EXPERIMENT 2						
Negative control	–		5.3	100	1	1.9
	+		5.0	100	2	4.0
Positive control 1,2,3,4-Diepoxybutane	–	0.04	3.7	70	790	2135
Decamethrin	–	1.0	5.0	94	4	8.0
	–	2.0	5.4	102	9	16.7
	–	3.0	5.5	104	4	7.3
	–	4.0	5.1	96	2	3.9
	–	5.0	4.4	83	2	4.6
	+	1.0	6.1	122	1	1.6
	+	2.0	5.3	106	2	3.8
	+	3.0	5.6	112	2	3.6
	+	4.0	4.8	96	4	8.3
	+	5.0	4.1	82	4	9.8

1976), particularly with regard to the profuse salivation and convulsive movements.

Decamethrin was not mutagenic when examined in several microbial strains and was not teratogenic in the rat or mouse at doses that produced obvious signs of maternal toxicity. The significance of the increased incidence of supernumerary ribs observed in the mouse experiment is not known at this time. Kimmel and Wilson (1973) have expressed the opinion that supernumerary ribs could be regarded as an indication of teratogenic potency at some higher dosage, but the degree of maternal toxicity present at the doses utilized in this study precludes the testing of that hypothesis. No similar effects were observed in the rat fetuses. Neonatal weight gain was reduced subsequent to perinatal treatment, but other signs of morphological or behavioral development were not. Since this effect was not present at birth and dissipated after weaning, it is probable that this was a compound-induced lactational effect.

Thus, decamethrin is a highly toxic pyrethroid ester. The developmental stages of the rat and mouse do not appear to be sensitive to the toxic effects of decamethrin exposure, and the mutagenic hazard is low as evidenced by microbial bioassay procedures.

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